



NAMRU-SA MISSION

"To conduct medical, dental, and directed energy biomedical research, which focuses on ways to enhance the health, safety, performance, and operational readiness of Navy and Marine Corps personnel and addresses their emergent medical and dental problems in routine and combat operations."

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Joint NAMRU-SA, Army ISR Project Recognized at 2013 MHSRS Annual Conference

LT Saima Raza, PsyD, MSC, USN

Dr. John Simecek, DDS, Director, Craniofacial Health and Restorative Medicine, Naval Medical Research Unit San Antonio (NAMRU-SA), and his Army collaborators from the Dental and Trauma Research Detachment, Army Institute of Surgical Research (ISR), were recognized at the 2013 Military Health System Research Symposium (MHSRS) Annual Conference. Their poster on *Incidents and Risk Factors on Dental Disease and Non-Battle Injury among U.S. Army Components in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF)* took the Silver Award in the poster competition, out of 280 posters submitted.

The poster highlighted the findings of a study on evaluating risk factors in dental emergencies not attributed to combat. The study found that approximately 15% of military personnel will experience a

Dental Disease-Non-battle Injury (D-DNBI) during a one year deployment.

The D-DNBI data was available for OIF from May 2009 to December 2011, while OEF data covered the period of July 2010 to December 2012. A total of 31,659 entries were included in the study.

The study analyzed data entered using the Corporate Dental Application module, which will be deployed in the Navy dental care system in the near future. Data from the study may be used to develop a model that will more accurately predict the number of D-DNBI incidents that can be expected during a deployment. Improving dental readiness could significantly increase Individual Medical Readiness and enhance operational capabilities. Future studies are planned to determine individual risk factors, develop new diagnostic methodologies, and develop D-DNBI predictive models which can be used to decrease D-DNBI cases.



Dr. John Simecek, left, MAJ Paul Colthirst, center, and COL Philip DeNicolo, right, display their winning poster at the Army Institute of Surgical Research entrance. The poster was recognized at the 2013 Military Health System Research Symposium Annual Conference.

Collaborative Efforts Improve Maxillofacial Osteogenesis Understanding

Rene Alvarez, PhD

A collaboration between Naval Medical Research Unit San Antonio (NAMRU-SA) and US Army Dental and Trauma Research Detachment (USADTRD) has been established to research the underlying mechanisms of maxillofacial bone regeneration and identification of specific infections often associated with maxillofacial injuries.

The study, titled *Evaluation of the Immune Response Associated with Maxillofacial Bone Regeneration and Wound Healing in a Porcine Model of Maxillofacial Osteogenesis* was initiated on June 17, 2013. The long-term goal of this research is the identification of immune biomarkers that would act as “early identifiers” for infection, the most significant issue facing successful maxillofacial bone regeneration.

Warfighter Personal Protective Equipment has improved significantly over the last ten years, leading to fewer torso and limb injuries, but leaving the head and neck region extremely vulnerable. The most common injury experienced after exposure to improvised explosive devices, mortars, rocket-propelled grenades, and gunshots while wearing protective gear is severely fractured or obliterated bones in the maxillofacial area. Unfortunately, there is a limited understanding of the osteogenesis process in the maxillofacial area, which differs significantly from long bone healing. However, one key parameter appears to be the role of inflammation associated with the immediate wound, and more importantly, with the kinetics of bone regeneration.

This collaborative effort

aims to evaluate the foundational profile of the inflammation response associated with maxillofacial osteogenesis at both the protein and mRNA levels, which will serve as the basis of biomarker identification. The study's findings will be utilized to improve both the development of novel osteogenesis therapeutics and improvement in standard of care for our wounded warriors.

This project leverages the extensive expertise of USADTRD with maxillofacial trauma models and NAMRU-SA's expertise and capabilities in immunodiagnostics and inflammation.



The joint team members of Naval Medical Research Unit San Antonio and US Army Dental and Trauma Research Detachment are shown here in a recent team photo in front of the Battlefield Health and Trauma building at Joint Base San Antonio Fort Sam Houston.

NAMRU-SA Gains Capabilities With New Spectrometers

LT Saima Raza, PsyD, MSC, USN

Naval Medical Research Unit San Antonio (NAMRU-SA) has recently completed installation and training on two newly acquired instruments with the latest groundbreaking analytical capabilities; an Atomic Absorption (AA) Spectrophotometer with a Vapor Generation Accessory (VGA) attachment and a High Performance Liquid Chromatography Triple Quadrupole Mass Spectrometer (HPLC-MS/MS).

The AA Spectrophotometer will provide a complete characterization of metals in dental wastewater and will provide data for many other NAMRU-SA projects such as measurements of the concentrations of silver, gold, or iron nanoparticles currently being assessed for their effectiveness in antibiotic resistance infection. The AA is equipped with a VGA attachment that increases its sensitivity to Hg, arsenic, selenium and a range of other hydride-forming elements. The ability to detect Hg at the sub-part per billion level will ensure NAMRU-SA/ Navy Medicine is successful meeting EPA requirements for mercury abatement within Medical Treatment Facilities.

The HPLC-MS/MS makes it possible to characterize molecules that are present in dental wastewater at extremely low concentrations. The HPLC-MS/MS not

only provides detection capability, but an understanding of the molecular state of a substance. The characterization of a molecule is of even greater importance than simply a basic analysis of the metals present in the analyte. For example, metallic Hg is fairly safe, and when bound with other metals in amalgam, is practically inert. However, the monomethylmercury(II) cation $[\text{CH}_3\text{Hg}]^+$ found in fish is one of the most poisonous compounds known. The ability to analyze a range of substances at ultralow concentrations provides opportunities to evaluate current remediation techniques or to determine potentially harmful environmental conditions prior to escalation to toxic levels.

Additionally, the HPLC-MS/MS will enhance other NAMRU-SA research. The instrument's capabilities include the measurement of biomarkers, growth factors, toxins, pesticides and much more, in human tissues and in environmental samples.

In summary, these new instruments will provide NAMRU-SA with the enhanced capability to identify various elements and molecules in multiple ongoing and future research areas that focus on ways to enhance the health, safety, performance, and operational readiness of Navy and Marine Corps personnel.

Dr. Wayne Deutsch, Mercury Abatement Program Manager, demonstrates the features of the Atomic Absorption spectrophotometer. Currently, the device is being used to analyze the effectiveness of filters used in Navy dental clinics to remove mercury and other dental wastewater contaminants.



NAMRU-SA Presentations & Products

Alvarez, R., (2013). Rapid Identification of Bacterial Pathogens of Military Interest Using Surface-Enhanced Raman Spectroscopy, Military Health System Research Symposium, Fort Lauderdale, FL.

Cestero, R. F., (2013). Independent Predictors of Mortality in Shock: An Analysis of 1703 Hypotensive Combat Casualties, American Association for the Surgery of Trauma, San Francisco, CA.

Cestero, R. F. and Song, B. K., (2013). The Effect of Hemostatic Dressings in a Subclavian Artery and Vein Transection Porcine Model, Military Health System Research Symposium, Fort Lauderdale, FL.

Colthirst, P., Berg, R., DeNicolo, P., & Simecek, J., (2013). Operational cost analysis of dental emergencies for deployed US Army personnel during Operation Iraqi Freedom. *Military Medicine*, 178, 427-431.

Cundin, L., Elliot, W., Barsalou, N., Voss, S., (2012). A Compact Analytic Solution Describing Optoacoustic Phenomenon in Absorbing Fluid, arXiv.org, Cornell University.

DeSilva, M., (2013). Microelectrode Array-Based Neurotoxin Biosensor with Chick Embryo CNS Neurons, International Conference on Biomolecular Engineering, Fort Lauderdale, FL.

DeSilva, M., (2013). The Effects of Magnetic Nanoparticles on Human Corneal Epithelial Cell Viability, The Tissue Engineering and Regenerative Medicine International Society, Atlanta, GA.

Hwang, Y., Ramalingam, K., Bienek, D., Lee, V., You, T., & Alvarez R., (2013). Antimicrobial activity of nanoemulsion in combination with cetylpyridinium chloride in multidrug-resistant *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy*, 57, 3568-3575.

Laccabue, M., Ahlf, R., & Simecek, J., (in press). Frequency of restoration replacement in posterior teeth for US Navy and Marine Corps personnel. *Operative Dentistry*. doi: <http://dx.doi.org/10.2341/12-406-C>

Rall, J., Cox, J. M., Songer, A., Comeaux, J. A., Estep, J. S., Cestero, R. F., Ross, J. D., (2012). Comparison of Novel Hemostatic Gauzes to Quickclot Combat Gauze in a Standard Swine Model of Uncontrolled Hemorrhage, Military Health System Research Symposium, Fort Lauderdale, FL.

Sheppard, F. R., (2013). Traumatic Hemorrhage in Combat Deaths, DoD Hemorrhage and Resuscitation, R&D Steering Committee, Ft. Detrick, MD.

Simecek, J. W., (2013). Incident and Risk Factors of Dental Disease Non-Battle Injury Among US Army Components in OIF and OEF, Military Health System Research Symposium, Fort Lauderdale, FL.

Song B. K., William H. N., Moon-Massat P. F., Auker C. R., McCarron R., Pittman R. N., (2013). Effects of Top-Loading a Zero-Link Bovine Hemoglobin, OxyVita, on Systemic and Microcirculatory Variables, *Military Medicine*, Vol. 178(5), 570-577.

Song, B. K., (2013). Effects of Top-Loading a Third Generation Oxygen Therapeutic on Systemic and Microcirculatory Parameters, Presented at the Military Health System Research Symposium, Fort Lauderdale, FL.

Vivekananda, J., Millenbaugh, N., (2013). Nucleic Acid Aptamers: An Emerging Class of Therapeutics to Neutralize Bacterial Toxins, Military Health System Research Symposium, Fort Lauderdale, FL.

Identification Of Bacterial Agents Will Aid Combat Casualties

Rene Alvarez, PhD

Naval Medical Research Unit San Antonio (NAMRU-SA) Immunodiagnostics Department conducted a study to evaluate the utilization of Surface Enhanced Raman Spectroscopy (SERS) for detection and generation of "molecular fingerprints" of military relevant infectious organisms.

According to the Joint Theater Trauma Registry, approximately 25% of combat casualties are found to develop an infection, with the rate approaching 50% in wounded warriors requiring intensive care treatment. Infections are commonly associated with methicillin-resistant

Staphylococcus aureus, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella Pneumoniae*.

These infections result in extensive medical complications and negative outcomes for patients. Antibiotic prophylaxis has been a standard of care, however successfully treating infection depends on a complete understanding of the bacteria present in and around the wound. Therefore, a rapid and sensitive means of diagnosing bacterial agents is critical for accurate and successful infection care.

A total of sixteen isolates including six *A. baumannii*, four *S. aureus*, three *K. pneumoniae*, and three *P. aeruginosa* strains were evaluated by SERS to determine uniqueness and commonalities of each spectra. NAMRU-SA's data demonstrate that SERS could generate unique "molecular fingerprints" for these organisms in 15-30 seconds.

All data were confirmed by utilization of organism specific quantitative real-time PCR amplification. These data demonstrate the valuable potential for the use of SERS-based platforms for rapid detection of microorganisms.

Blood Substitutes Show Promise for Battlefield Resuscitation

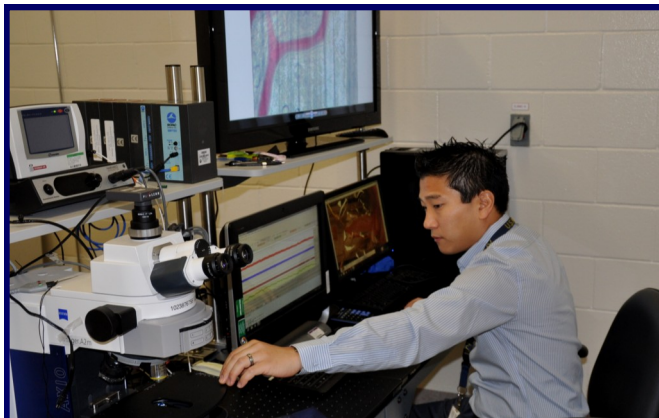
Bjorn Song, PhD

Currently, hemorrhage is one of the most preventable forms of military casualty, with survival being dependent on prompt hemorrhage control and restoration of both fluid volume and oxygen carrying capacity. Oxygen therapeutics (OTs), also known as blood substitutes, have several attributes that could make them an ideal choice for pre-hospital resuscitation. Blood substitutes behave as plasma expanders and have an oxygen carrying capacity that is greater than crystalloids and colloids. Unlike blood, OTs can be stored at room temperature, and have a shelf life of up to five years. Since OTs are extracellular agents, they have universal applicability and do not require blood typing. From a public health standpoint, there is a significantly lower risk of the transmission of blood borne diseases. However, reports of undesirable vasoactivity are believed to be associated with severe complications that have halted several clinical trials.

The most recently developed OTs have been engineered to be less vasoactive. Naval Medical Research Unit San Antonio (NAMRU-SA) is currently investigating these new forms of OTs. In an effort to down-select from among several varieties of OTs, a top-load in vivo model was utilized in the first phase, in which normovolemic rats were given serial infusions to establish a dose response relationship as well as evaluate the cumulative dose effect. Vasoactivity was

measured systemically, i.e., blood pressure, via an arterial catheter, while it was also monitored at the microcirculation level where vessel diameter changes were recorded. However, other physiological parameters were also measured to assess the efficacy of these OTs, such as cardiac output and functional hemoglobin content.

There were no significant adverse effects reported, and the tested OTs have been recommended for evaluation in a more clinically relevant hemorrhagic shock model. Further research is warranted as there is a demand to identify a safe and beneficial pre-hospital oxygen carrying resuscitation solution that could save the lives of our military personnel in austere and combat environments.



Dr. Bjorn Song conducts a test analyzing oxygen therapeutic agents as part of his research.

Testing of Hemostatic Gauzes Works to Improve Battlefield Injuries

Rene Alvarez, PhD

Uncontrolled hemorrhage is one of the leading causes of death among warfighters and has been associated with 90% of the potentially survivable deaths during recent conflicts. New point-of-care treatments that can reduce or stop bleeding could lead to a reduction of pre-hospital mortality through better hemostatic control. The addition of an occlusive dressing applied with hemostatic gauzes could compliment the standard of care and lead to better outcomes. Scientists at Naval Medical Research Unit San Antonio (NAMRU-SA) have performed a series of experiments aimed at evaluating the efficacy of newly developed hemostatic gauzes together with an occlusive dressing using a standardized animal model of hemorrhage.

This model was developed by a committee of medical experts to reproducibly test hemostatic

products in a model representing injuries obtained in theatre. Metrics including survival, ability to achieve hemostasis, and amount of blood shed during the observation period were recorded and compared among test gauzes. In addition to blood loss, hemodynamic properties, such as blood pressure and heart rate; and biochemical properties, such as hemoglobin concentration, pH, and fibrinogen concentration, were also compared. Initial studies on four novel gauzes indicated that differences among the newly developed dressings were minimal when compared with QuikClot® Combat Gauze™, the standard of care currently approved by the Committee on Tactical Combat Casualty Care.

NAMRU-SA is presently evaluating other novel products in the hemorrhage and resuscitation arena aimed at reducing the amount of potentially survivable deaths in current and future conflicts.

Laser-Induced Antimicrobials May Eradicate Combat-Related Pathogens

Nancy J. Millenbaugh, PhD

A project ongoing at Naval Medical Research Unit San Antonio (NAMRU-SA) seeks to develop a new treatment for methicillin-resistant *Staphylococcus aureus* (MRSA) maxillofacial infections that is an alternative to conventional antibiotics.

Combat wounds often present with a high rate of contamination from the environment and therefore quickly become infected. Treatment of combat-related wound infections is complicated by the fact that a significant percentage are caused by multi-drug resistant bacteria such as MRSA.

Maxillofacial, eye, head, and neck regions are particularly vulnerable to sustaining serious battlefield injuries from improvised explosive devices.

Along with conventional multi-drug resistance, a challenging factor for successful treatment of infections is the formation of bacterial biofilms within the wound that act as a physical barrier against antibiotics and the host immune system which impairs wound healing.

New approaches being developed at NAMRU-SA

that can overcome both traditional multi-drug resistance and biofilm resistance mechanisms include pulsed laser irradiation combined with energy absorbing nanoparticles targeted against MRSA. Researchers hope this will significantly decrease the viability of the bacteria in planktonic and biofilm forms via generation of photothermal processes that induce physical damage to the bacterial cell wall and the biofilm matrix. This approach could prove effective at eradicating pathogens regardless of their level of antibiotic resistance. If successful, this new treatment regime will give health care providers an alternative, non-pharmaceutical approach to treating combat wounds.

Dr. Nancy Millenbaugh, Research Chemist, center, monitors a laser test being conducted by Mr. Don Marchello, Electronic Technician, left, as Mr. Jonathan Baskin, Research Biologist, right, examines bacterial samples.



Commanding Officer's Corner

CAPT Rita G. Simmons, PhD, MSC, USN

As we complete this edition of the *Science Journal*, some of the difficult budget realities of this, and possibly future years, are coming to light. NAMRU-SA has used these challenges as opportunities to extend areas of collaboration and to review our research portfolio for relevance and efficiency. Over the course of the past year, we have built joint collaborative teams with our academic and DoD partners and kept our commitment of executing gap-driven research and delivering relevant products to the DoD medical community. In addition, after our review and some mission-driven adjustments, we can confidently state that we currently possess more capabilities and resources to perform Navy, and joint research, at a greater value for our DoD warfighters than in previous years. In a tight budget environment, the ability to leverage talents and capabilities has been more crucial than ever before.

As the Navy and DoD medical communities continue to improve battlefield care, our command is well-poised to individually and collaboratively answer critical biomedical research questions at hand. Our diverse team provides, if you will, the best 'bang for the buck' in addressing many different research areas within combat casualty care and craniofacial health. For example, some of our most recent work, which may not be widely known, has been investigating possible blood substitutes, developing ways to prevent post-surgical cranial infections through antibacterial coatings,

studying biomarkers and how they can be used to detect viral and bacterial infections, and evaluating procedures to rapidly detect microorganisms. This research represents cutting-edge techniques and potential solutions to biomedical conditions that often hinder our military personnel. For those who have not had the opportunity to visit the new facilities at NAMRU-SA, I would highly encourage you to do so. It is difficult to verbally paint an adequate picture of the vast capabilities and fully explain the resident expertise. Seeing is definitely believing!

Without a doubt, the current budget challenges represent some very demanding times. Challenges, however, allow us to step up and overcome, and I believe, have made the team at San Antonio work even harder to ensure our products remain pertinent to the Navy Medicine mission, and that they are quickly transitioned to our medical community for enhanced care on the battlefield. Even in this uncertain fiscal environment, I know that great things lie ahead for our command, Navy Medicine, and the DoD.



**CAPT Rita G. Simmons
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Taking the Helm of Navy Medicine's Combat Casualty & Craniofacial Health Research

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